

Amendments to the Claims:

1. (withdrawn) A modified cytochrome P450 monooxygenase which, in comparison with the wild-type enzyme, shows an altered substrate profile in the terminal and/or subterminal enzymatic hydroxylation of aliphatic carboxylic acids, owing to site-specific mutagenesis of its substrate binding region.
2. (withdrawn) A monooxygenase as claimed in claim 1, which is derived from cytochrome P450 monooxygenases of bacterial origin.
3. (withdrawn) A monooxygenase as claimed in claim 2, which is derived from *Bacillus megaterium* cytochrome P450 monooxygenase BM-3 with an amino acid sequence in accordance with SEQ ID NO:2, which has at least one functional mutation in one of the following amino acid sequence regions: 24-28, 45-51, 70-72, 73-82, 86-88, 172-224 and 352-356, with the proviso that, if the enzyme carries the mutation FS7A, more than one of these regions is mutated.
4. (withdrawn) A monooxygenase as claimed in claim 3, which comprises at least one functional mutation in the amino acid sequence regions 86-88 and 172-224.
5. (withdrawn) A monooxygenase as claimed in claim 4, which comprises at least one of the following amino acid substitution patterns:
 - a) F87V;
 - b) F87A L188K;
 - c) F87V L188K;
 - d) F87A L188 KA74G;
 - e) F87V L188K A74G;
 - f) F87A L188K A74G R47F;

- g) F87V L188K A74G R47F;
 - h) F87A L188K A74G R47F V26T; or
 - i) F87V L188K A74G R47F V26T;
- and functional equivalents thereof.
6. (withdrawn) A monooxygenase as claimed in claim 3, which comprises a single amino acid substitution from amongst the following:
- a) V26T,
 - b) R47F,
 - c) S72G,
 - d) A74G,
 - e) F87V,
 - f) L188Z, where Z is an amino acid selected from amongst K, R, W, Q, N, G, A and S, and
 - g) M354T;
- and functional equivalents thereof.
7. (withdrawn) A nucleic acid sequence encoding a monooxygenase as claimed in claim 1 and the complementary nucleic acid sequence thereof.
8. (withdrawn) An expression construct comprising, under the genetic control of regulatory acid sequence, an encoding sequence which encompasses a nucleic acid sequence as claimed in claim 7.
9. (withdrawn) A vector which encompasses at least one expression construct as claimed in claim 8.
10. (withdrawn) A recombinant microorganism which has been transformed with at least one

vector as claimed in claim 9.

11. (withdrawn) A microorganism as claimed in claim 10, selected from amongst bacteria of the genus *Escherichia*.
12. (currently amended) A process for the enzymatic production of subterminally hydroxylated aliphatic carboxylic acids, which comprises
 - a1) culturing a recombinant microorganism which has been transformed with a vector which encompasses an expression construct comprising, under the genetic control of regulatory nucleic acid sequences, a sequence which encompasses a nucleic acid sequence encoding a monooxygenase which is derived from *Bacillus megaterium* cytochrome P450 monooxygenase BM-3 with an amino acid sequence comprising containing SEQ ID NO:2 containing one functional mutation in each of amino acid sequence positions 87 and 188 and, optionally, at least one additional functional mutation in one of amino acid sequence positions 26, 47, 72, 74 and 354, wherein:
 - Phe 87 is replaced by at Val, Ala or Leu;
 - Leu 188 is replaced by Asn, Gln, Arg, Lys, Ala, Gly, Ser or Trp;
 - Ala 74 is replaced by Val or Gly;
 - Arg 47 is replaced by His, Tyr or Phe;
 - Val 26 is replaced by Ser or Thr;
 - Ser 72 is replaced by Ala, Leu, Ile or Gly; or,
 - Met 354 is replaced by Ser or Thr,

~~which has a functional mutation in the amino acid sequence region 86-88 and optionally at least one further functional mutation in one of the following amino acid sequence regions: 24-28, 45-51, 73-82 and 172-224 with the proviso that, if the enzyme carries mutation F87A, more than one of these regions is mutated,~~

which functional mutation in comparison with the wild-type enzyme, results in an altered activity or regioselectivity in the subterminal enzymatic hydroxylation of an aliphatic C₈-C₁₂-carboxylic acid, whereby culturing is performed in the presence of a culture medium which contains at least one hydroxylatable C₈-C₁₂-carboxylic acid or a derivative thereof, said derivative being selected from an alkyl ester, an amide or an anhydride of the at least one hydroxylatable C₈-C₁₂-carboxylic acid; or

- a2) incubating a reaction medium containing at least one hydroxylatable C₈-C₁₂-carboxylic acid or a derivative thereof, said derivative being selected from an alkyl ester, an amide or an anhydride of the at least one hydroxylatable C₈-C₁₂-carboxylic acid with a modified monooxygenase as defined above, and
- b) isolating the resulting hydroxylated products ~~product~~ from the medium.

13. (canceled)

14. (currently amended) A method as claimed in claim 12, wherein the at least one hydroxylatable carboxylic acid is a C₈-C₁₂-monocarboxylic acid or the derivative thereof, and the monooxygenase comprises at least one of the following amino acid substitution patterns in ~~an amino acid sequence of~~ SEQ ID NO:2:

- a) F87V;
- b) F87A and L188K;
- c) FS7V and L188K;
- d) F87A, L188K and A74G; F87A-L188K and A74G;
- e) F87V, L188K and A74G; F87V-L188K and A74G;
- f) F87A, L188K, A74G and R47F; F87A-L188K-A74G and R47F;
- g) F87V, L188K, A74G and R47F; F87V-L188K-A74G and R47F;

Application No.: 10/031,695

Inventor: HAUER et al.

Reply to Office Action of July 27, 2006 And Notice of March 27, 2007

Docket No.: 51241

- h) F87A, L188K, A74G, R47F and V26T; or
F87A L188K A74G R47F and V26T; or
- i) F87V, L188K, A74G, R47F and V26T. F87V L188K A74G R47F and V26T.
15. (canceled)
16. (previously presented) A method as claimed in claim 12, wherein the enzymatic production is carried out in the presence of an electron donor or a reduction equivalent.
17. (currently amended) A method as claimed in claim 16, wherein the electron donor or the reduction equivalent is selected from the group consisting of NADH, NADPH and Zn/CO(III) sephulchrate.
18. (currently amended) The process of claim 12, wherein the monooxygenase comprises at least one functional mutation in the amino acid sequence of region 86-88 and at least one functional mutation in the amino acid sequence of region 172-224.
19. (canceled)